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ORIGINAL RESEARCH ARTICLE

Glutamine-Driven Metabolic Adaptation to COVID-19 Infection

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Abstract

Background COVID-19 is known to be transmitted by direct contact, droplets or feces/orally. There are many factors which determines the clinical progression of the disease. Aminoacid disturbance in viral disease is shown in many studies. İn this study we aimed to evaluate the change of aminoacid metabolism especially the aspartate, glutamine and glycine levels which have been associated with an immune defence effect in viral disease.

Methods Blood samples from 35 volunteer patients with COVID-19, concretized diagnosis was made by oropharyngeal from nazofaringeal swab specimens and reverse transcriptase-polymerase chain reaction, and 35 control group were analyzed. The amino acid levels were measured with liquid chromatography-mass spectrometry technology. Two groups were compared by Kolmogorov–Smirnov analysis, Kruskal–Wallis and the Mann–Whitney *U*. The square test was used to evaluate the tests obtained by counting, and the error level was taken as 0.05.

Results The average age of the patient and control group were 48.5 ± 14.9 and 48.8 ± 14.6 years respectively. The decrease in aspartate ($p = 5.5 \times 10^{-9}$) and glutamine levels ($p = 9.0 \times 10^{-17}$) were significiantly in COVID group, whereas Glycine ($p = 0.243$) increase was not significiant.

Conclusions Metabolic pathways, are affected in rapidly dividing cells in viral diseases which are important for immun defence. We determined that aspartate, glutamine and glycine levels in Covid 19 patients were affected by the warburg effect, malate aspartate shuttle, glutaminolysis and pentose phosphate pathway. Enteral or parenteral administration of these plasma amino acid levels will correct the duration and pathophysiology of the patients' stay in hospital and intensive care.

Keywords Warburg effect · Glutaminolysis · Pentose phosphate pathway · Malate aspartate shuttle · Aspartate · Glutamine · Glycine · Amino acids

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Introduction

In this study, we would like to discuss the metabolic response to COVID-19 infection from the perspective of cellular pathology, which received little attention after Rudolf Virchow [1]. The disease progresses with COVID-19-induced cell death of alveolar cells, depending on the viral strain.

Metabolic Adaptation to SARS-CoV-2 İnfection

SARS-CoV-2, which has recently been accepted as a new coronavirus infection that causes severe acute respiratory syndrome in many different geographical regions, has been declared as one of the emerging infectious diseases by the World Health Organization. This novel coronavirus was classified as SARS-CoV-2 by the International Committee on Virus Taxonomy and was first seen in Wuhan, Hubei Province of China[2].

New variants were quickly identified after the original Covid virus found in Wuhan in December 2019. Alpha variant: in UK in September 2020. Beta variant: in South Africa in May 2020. Gamma variant: in Brazil in November 2020. Epsilon variant: in the US in March 2020. Zeta variant: in Brazil. Eta variant in multiple countries in December 2020. Theta variant: in the Philippines in January 2021. Iota variant: in the US in November 2020. Kappa variant: in India. Delta variant: was also found in India. SARS-CoV-2 is a disease with low pathogenicity and a high risk of transmission [2, 3].

According to the data of the World Health Organization; As of 13 May 2020, there were 4,170,424 (287,399 deaths) confirmed cases and 174,061,995 (3,758,560 deaths) confirmed cases as of 10 June 2021 [4, 5].

COVID-19 is known to be transmitted from animals to humans and from humans to humans by direct contact, droplets, or feces/orally [6, 7]. The incubation period of COVID-19 typically ranges from 2 to 14 days (98% of patients), with an average of 5 days. But cases with incubation periods of up to 24 days have also been reported.

Typically, the duration from the onset of infection to the development of severe disease (including hypoxia) is 1 week [8].

Clinical symptoms observed in COVID-19 patients include fever, dry cough, myalgia, fatigue, headache, drowsiness, anorexia, while symptoms such as diarrhea, hemoptysis, and shortness of breath are rarer [9, 10]. It is especially serious in the elderly, hypoxic patients with extensive lung involvement, and in individuals with comorbidity (cardiovascular diseases, diabetes mellitus, chronic lung disease, hypertension, and cancer) [11]. COVID-19 causes severe symptoms in some patients, while others are completely asymptomatic.

There is no known treatment method specific to COVID-19 other than symptomatic treatment. Although current symptomatic treatment protocols seem beneficial for some patients, they have not been beneficial for many patients and deaths continue. Although attempts were made to standardize symptomatic treatment protocols, it failed due to individual differences in patients.

Everyone knows very well that it is the cells attacked by viruses that are intended to be treated. Moreover, the human organism has a immune system that will destroy the viruses that attack its cells, and it is even more effective than the known drugs. Another fact is that many of the symptoms seen in COVID-19 patients, and even cytokine storms, are the result of exaggerated attack strategies of the immune system.

The body's defense against viral attacks is not limited to the immune responses. Infection is a stressor, in Hans Selye's words [12]. It is a new situation to the body. Adaptation of the organism to this new situation requires a heavy metabolic expense. However, the virus uses the host's metabolic possibilities to make copies of itself. In this case tissues have to enter into serious competition for the amino acids and energy they need to repair themselves or replace dead cells. The body's metabolic response to COVID-19 infections has been partially demonstrated in non-targeted metabolomics studies [13]. We had been examined the metabolic responses to Crimean-Congo hemorrhagic fever virus infections and presented our findings in our previous articles [14]. Similarly, we analyzed the metabolic response to COVID-19 infections in terms of amino acid metabolism and wanted to bring it to your attention.

Alterations in Plasma Amino Acids May be an Indicator of Metabolic Adaptation to SARS-CoV-2 Infection

Main question, if the distruption of amino acid metabolism a kind of metabolic adaptation to COVID-19 infection or excessive amino acid consumption? In viral infections replacement of damaged or dead cells takes place through self-renewal and differentiation of healthy stem cells.

Although the intervention of the immune system is the main determinant in the combat against viral infections, the repairing process is possible with the supply of essential elements such as fats, sugar, nucleotides and amino acids.

Amino acids are not only the building blocks for cell repair and/or proliferation, but they are also indispensable raw materials for energy metabolism, synthesis of nucleotides, neurotransmitter, peptide hormones, and enzymes [15, 16].

The use of changes in plasma amino acid levels as a biomarker in the diagnosis and treatment of many diseases is not a new practice [17–19].

Recently, some research has been realized on how the cellular amino acid metabolism of the host is altered in viral infections. Viruses are obligate intracellular parasites that take over the cellular metabolism of the host for their own replication [20]. Metabolic intermediates that occur in many viral infections, including human cytomegalovirus, herpes simplex virus, and hepatitis B virus, have been shown to play an important role in disease progression and severity [21, 22].

Our aim was to see how the amino acid metabolism of individuals infected with SARS-Cov2 changed and to understand whether the changes were a protective adaptation or a result that led to burnout. For this purpose, amino acid metabolism was investigated in SARS-CoV-2 infected patients who applied to the emergency department of Sivas Cumhuriyet University Hospital.

Materıal and Methods

In this study, 35 blood samples from volunteer patients with COVID-19, concretized diagnosis was made by oropharyngeal from nazofaringeal swab specimens and reverse transcriptase-polymerase chain reaction (RT-PCR) test, who has attended Sivas Cumhuriyet University Medical Faculty Emergency Department or State Hospital pandemic clinics were collected. This research is derived from the study "determination of changes in plasma Amino acid level in COVID-19 patients", which was approved by Sivas Cumhuriyet University interventional Ethics Committee with the number 2020–04/02.

Patients Group

Exclusion/İnclusion Criteria

Those with alcohol and substance use, acute or chronic disease (such as Diabetes Mellitus, hypertension, chronic kidney failure, heart failure, liver damage), autoimmune disorders, or with a focal or systemic infection were not included in the study. Patients who required intensive care and/or died were not included in the study. Our study's inclusion criteria included all in-patients who had a positive real-time polymerase chain reaction (RTPCR) test, were older than 18 years and were hospitalized in the clinics.

Healthy Group

Exclusion/İnclusion Criteria

The exclusion criterias for the control group were also the same like the patients group. A control group was created from blood samples taken from 35 healthy volunteers who did not have any systemic disorders and were similar to the patient group in terms of gender and age.

Samples

About 5 ml of venous blood samples were taken from patients and healthy volunteers into BD Vacutainer® PST™ Tubes contain spray-coated lithium heparin and a gel for plasma separation. They are used for plasma determinations in chemistry.

All blood samples were centrifuged for 10 min at 4 °C and 4100 rpm in a centrifuge (Nüve NF 800R; Ankara, Turkey). The resulting plasma was aliquoted into the eppendorf tubes and stored at − 80 °C until testing.

Finished the required number of patients in the study was reached, all the samples were defrosted and the amino acid levels were measured according to the method specified, at one time, liquid chromatography-mass spectrometry (LC–MS/MS) technology by using a quantitative amino acid analysis kit, Jasem laboratory systems and solutions; Istanbul, Turkey.

Statistical Analysis

In our study, the obtained data using SPSS (Ver:23.0) computer program for the evaluation of parametric test assumptions are satisfied when installing the data by Kolmogorov–Smirnov analysis of variance, analysis materiality as a result of the decision that makes the difference to find the group or groups when given binary comparison methods cannot be fulfilled when the assumptions of parametric test Kruskal–Wallis test was used. The Mann-Whitney *U* test was used for groups or groups that differed when the materiality decision was made as a result of the Test. The square test was used to evaluate the tests obtained by counting, and the error level was taken as 0.05.

Results

The average age of the patient group included in the study was 48.5 ± 14.9 years (19–69) and the average age of the healthy control group was 48.8 ± 14.6 years (19–74). 23 (65.7%) of the patients were male and 12 (34.3%) were female. In the healthy control group, 22 (62.9%) were male and 13 (36.1%) were female. There wasn't any statistically

significancy according to age (p = 0.936) and gender (p = 0.685) differences between the groups.Male patients (n = 23) accounted for 65.7% of the total cases, while female (n = 12) patients comprised 34.3%.

The amino acids and their derivatives, which are the subject of this article, were quantitatively measured in the heparinized plasma of the patients (n = 35) by LC–MS/MS method. Statistical differences detected in this broad plasma amino acid profile are shown in Table 1 and commented on in the discussion section of this article.

As can be seen in Table 1, plasma Alanine, arginine, argininosuccinic acid, asparagine, aspartic acid, glutamine, histidine, lysine, citrulline, ornithine, proline, and threonine levels were significantly ($P < 0.05$) decreased in COVID-19 positive cases. However, there was a statistically significant ($p < 0.05$) increase only in glutamate and methionine levels.

Discussion

Amino acids are essential building blocks for proliferating immune cells and even for all other cells. The human body's response to lethal virus attacks is not limited to the responses of immune cells alone. In reality, the immune response is only one part of the total battle against this virus. Supporting energy metabolism, availability of amino acids, inhibition of nutrition of virus-infected cell, metabolism of calcium in muscle, bone, repairing damaged tissues all are an integral part of this war. In this study, we planned to see whether the organism attacked by the COVID-19 virus could meet its vital needs and how it underwent a metabolic adaptation to survive.

In patients infected with COVID-19, plasma Alanine, Arginine, Arginosuccinic acid, Aspartate, Glycine, Histidine, Lysine, Citruline, Threonine, and Glutamine levels reduced, while Methionine and GABA levels had been elevated. If insufficient amino acid availability causes problems in nucleic acid and energy metabolism in COVID-19 patients, this could adversely affect the clinical course of the disease.

We think that the decrease in amino acid levels observed in this table is not due to a metabolic disorder or nutritional deficiency, but to excessive consumption (Table 1).

Indeed, Hortin et al. found a significant decrease in plasma amino acid levels also in HIV infections, which is a viral disease that causes burnout[23].

When we observe such a this severe reductions in amino acid levels, we tried to develop a hypothesis that could explain this decrease.

One of the hypotheses that could explain our observation might be that the virus also induces metabolic reprogramming in host cells, similar to the Warburg effect in cancer. In other words, the virus might want to reduce the host cells' ability to utilize oxygen and allow itself to reproduce under anaerobic conditions. Enhance mitochondrial depletion and reduce oxygen consumption could also mean that the function of immune cells working against the virus would be limited [24, 25].

Systems-based medicine approaches that support this view today have revealed the direct relationships between cell metabolism and viral infections [26, 27].

Although these studies are mostly about carbohydrate and energy metabolism, they also touch on amino acid metabolism. Serious links have been established between metabolic reprogramming of host cells and immune cells and severe tissue inflammation caused by severe acute respiratory syndrome-coronavirus 2, the etiologic agent of COVID-19 [28–30].

Does this disease force immune cells to anaerobic respiration even in an abundant oxygenated (aerobic) environment, or is anaerobic respiration a metabolic response necessary for survival in this respiratory airway disease? We couldn't help but ask these questions.

As it is known, at the beginning of the twentieth century, Otto Warburg showed that cancer cells consume glucose and produce lactate with high levels of anaerobic respiration even in the presence of oxygen (O_2). This unexpected process is known as "aerobic glycolysis" or "Warburg effect".

Due to the Warburg effect, the mitochondrial tricarboxylic acid (TCA) cycle is not fed naturally with acetyl-CoA converted from pyruvate, and in this case, the fuel that feeds the TCA cycle is glutamine, which can convert to α-ketoglutarate. Also, glutamine since it is a nitrogen source for nucleotide synthesis, consumption is very high. As the Warburg effect glutamine metabolism drives FAS and nucleotide synthesis simultaneously, in fact, it may be one of the key targets for virus replication as well as a cancer cell [31].

These theoretical approaches are consistent with our findings with glutamine depletion. The elevation of glutamate in our study was also very significant. As is known, glutamine also supports glutamate production and citrate synthesis by maintaining the functioning of the TCA cycle. Although citrate synthesis takes place in the mitochondria, it is also transferred to the cytoplasm and splits into acetyl-CoA and oxaloacetate. Oxaloacetate also acts as a precursor molecule for Threonine, Asparagine, Aspartate and later Arginine synthesis and even NO synthesis [31]. When we look at our own results, it is seen that the levels of Threonine, Aspartate, and Arginine levels are significantly reduced. Of course, we do not know whether this result is spent on the production of NO synthesis, which feeds the proinflammatory pathway. However, overproduction of

Table 1 Plasma amino acid levels of control group and COVID-19 patients

	CONTROL (n = 35)		COVİD-19 (n = 35)		P
	Mean ± SD μmol/L	Min.-Maks	Mean ± SD μmol/L	Min.-Maks	
Alanine (Ala)	659.5 ± 155	278–913	447 ± 113	249–643	9.8×10^{-9}
Arginine (Arg)	84.2 ± 48.2	34.0–243.9	53.6 ± 21.9	1.6–98.4	1.0×10^{-3}
Arginosuccinate	1.96 ± 1.67	0.12–7.5	1.03 ± 0.7	0.26–3.36	3.4×10^{-3}
Aspatate (Asp)	14.1 ± 8.9	3.4–42.3	3.8 ± 1.9	1.0–6.9	5.5×10^{-9}
Citruline	39.4 ± 12.5	20.5–70.1	27.8 ± 8.9	5.7–45.5	3.3×10^{-5}
Ethanolamine	5.69 ± 1.12	3.56–7.66	9.94 ± 2.35	4.27–16.13	2.21×10^{-14}
GABA	0.25 ± 0.05	0.11–0.34	0.28 ± 0.12	0.18–0.84	0.212
Glutamate (Glu)	102.1 ± 30.9	53.3–162.4	176.5 ± 57.8	68.5–301	4.4×10^{-9}
Glutamine (Gln)	814.4 ± 119.9	562–1062	489.6 ± 126.7	240–735	9.0×10^{-17}
Glycine (GlY)	316 ± 82.7	159–510	341 ± 95.4	169–587	0.243
Histidine (His)	134.9 ± 23.8	96.4–192	89.6 ± 21.7	56.2–129	5.5×10^{-12}
Isoleucine (Ile)	125.8 ± 41.9	75.1–280	111.5 ± 27.2	60.8–166.4	0.094
Leucine (Leu)	175.5 ± 50.5	112–341	167.5 ± 42.0	60.5–260.6	0.476
Lysine (Lys)	280.1 ± 54.2	174.7–419.8	206.4 ± 46.6	84.4–363.1	5.8×10^{-8}
Methionine (Met)	30.0 ± 7.3	16.8–48.1	35.3 ± 9.4	17.0–64.4	0.0109
Phanylalanine (Phe)	85.1 ± 9.3	61.7–98.4	80.5 ± 18.4	46.9–117.4	0.190
Threonine (Thr)	204.9 ± 55.8	88.2–343.9	159.9 ± 39.2	107.9–255.1	2.3×10^{-4}
Tryptophan (Trp)	76.6 ± 15.9	39–122	59.6 ± 13.2	28.6–83.6	6.99×10^{-6}
Tyrosine (Tyr)	113 ± 22.5	68.5–162.9	86.4 ± 14.6	54.3–115.4	1.39×10^{-7}
Valine (Val)	300 ± 63.3	198–475	261 ± 63.3	103–383	0.0113

Plasma Alanine, arginine, argininosuccinic acid, asparagine, aspartic acid, glutamine, histidine, lysine, citrulline, ornithine, proline, and threonine levels were significantly ($P < 0.05$) decreased in COVID-19 positive cases. However, there was a statistically significant ($p < 0.05$) increase only in glutamate and methionine levels

NO synthesis in patients with COVID-19 infection is a known fact [32].

Today, immuno-metabolic studies conducted not only for this virus but also with other viruses have come to the fore in understanding the pathophysiology and treatment of COVID-19 [24]. We don't know exactly what our findings in COVID-19 cases depend on. However, we believe that metabolic and immunometabolic alterations caused by viruses will shed light on us. Such immuno metabolic studies mostly support the Warburg effect, as presented below.

It is a fact that viruses trying to replicate themselves change the metabolic pathways of the host in line with their own needs in order to meet their bioenergetic and biosynthetic needs [33, 34]. Ramier et.al. reported a significant increase in glycolytic enzyme expression in HCV-infected cells [35]. Tay et.al. suggested that in the wild-type strain of Adenovirus 5 infection, glucose consumption and lactate production are increased, oxygen consumption is decreased, and the majority of glucose is converted to pentose phosphate pathway intermediates and nucleotides [36]. Recently, the Warburg effect has been clearly demonstrated in the early stages of influenza, cucumber mosaic and hepatitis C virus infections [37, 38]. One of the common findings of these studies is that the ATP obtained due to the Warburg effect is significantly less than that obtained by aerobic respiration, as expected.

It should not be forgotten that cells trying to multiply under the effect of Warburg they don't just use glucose to get energy and produce ATP. Intermediate products formed during glycolysis are also used in nucleotide, lipid and amino acid synthesis [39]. In addition, NADPH, which is formed as a result of pentose phosphate pathway reactions, is used especially in fatty acid synthesis and detoxification of oxidative radicals by reducing oxidized glutathione. In conclusion, the Warburg effect is a necessary mechanism for the rapid division of cells. However, it is not known yet clear how viruses use this mechanism to make themselves replicate [40].

In this study, the individual's previous metabolic disorders and nutritional status were not naturally a variable that we could control. We also know that looking at plasma amino acids alone is not enough to see the big picture. However, given the key role of amino acid metabolism, we

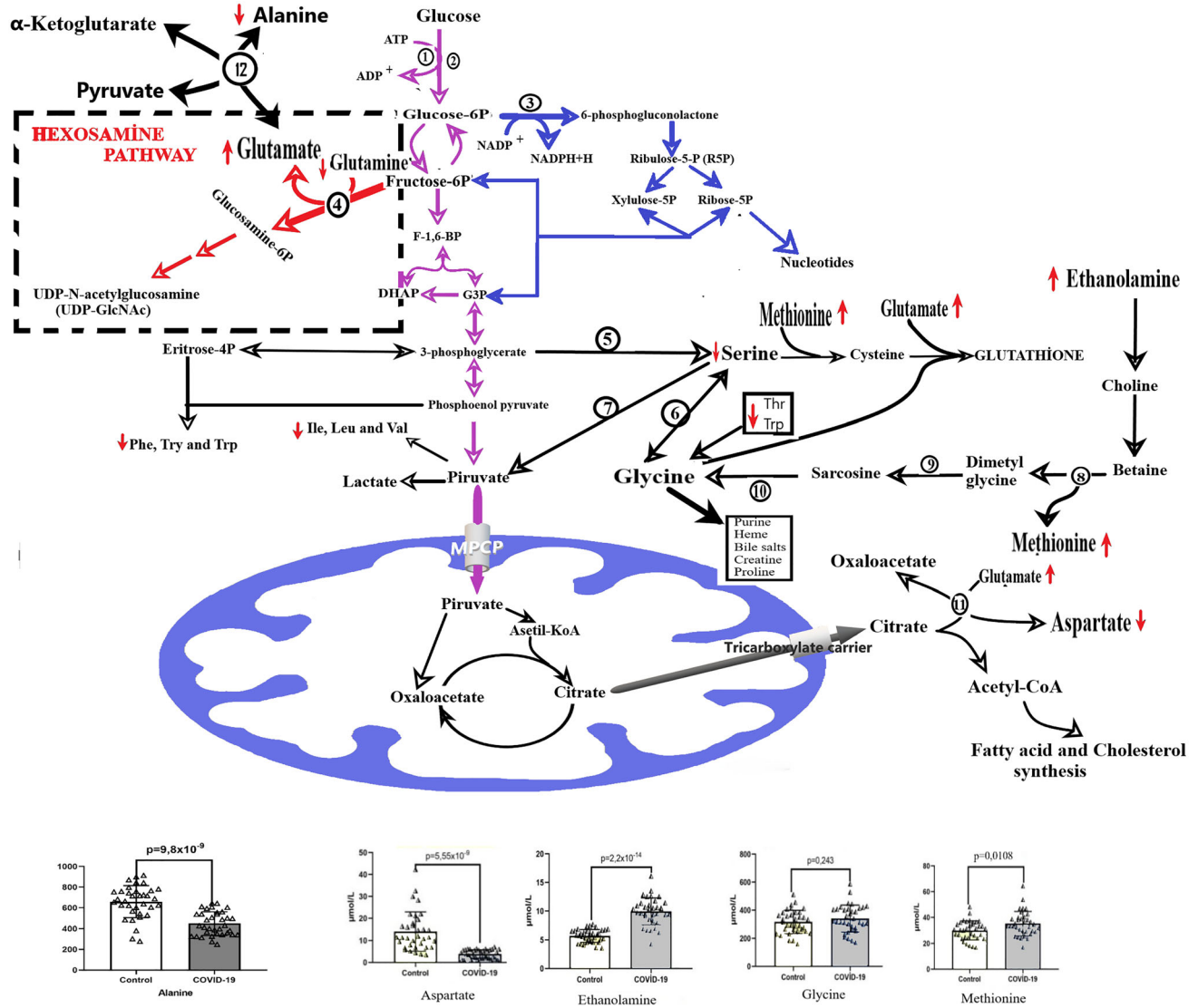

Fig. 1 Relationship with amino acid, Warburg effect, and what we determined in COVID-19 patients. During viral infections genome replication, the viral products accelerate glycolysis to support pathogen biosynthesis by providing glycolytic intermediates for the synthesis of nucleic acids, amino acids and lipids required for them, rather than energy. Mitochondrial carrier protein (MPCP). Notes: 1-Glucokinase, 2-Hexokinase, 3-Glucose-6-P-dehydrogenase, 4-Glutamine: fructose-6-Paminotransferase (GFAT), 5-Phosphoglycerate dehydrogenase, 6-Serine hydroxymetiltransferase, 7-Serine dehydratase, 8-Betaine transaminase, 9-Diethylglycine dehydrogenase, 10-Sarcosine dehydrogenase, 11-Aspartate transaminase (AST), 12-Alamine transaminase (ALT)

are confident that this is the first step to start. Although not enough, we tried to see the big picture by placing the amino acid changes we could detect in their places in the metabolic pathways like pieces of the puzzle. We wanted to simultaneously look at three separate metabolic pathways (Malate-Aspartate Shuttle, Glutaminolysis, and Hexosamine pathway) that are closely linked (Fig. 1).

What we could see was a general and severe amino acid depletion (Table 1). The virus had altered the ways of its energy metabolism to supply its building blocks and replicate itself. In the near future, our plan is to present our more in-depth review of the metabolism of non-essential amino acids synthesized from glycolytic intermediates along with the TCA cycle.

However, we think that glutamine depletion within the overall amino acid depletion we detect in Covid-19 infection may be the primary and starting point in solving this puzzle.

Glutamine, is the most abundant amino acid in plasma, is used as a source of energy, carbon, and nitrogen to support cellular homeostasis [41–43]. It is an indispensable nitrogen donor for the de novo synthesis of hexosamines such as purine, pyrimidine, non-essential amino acids, glucosamine and galactosamines [28, 29].

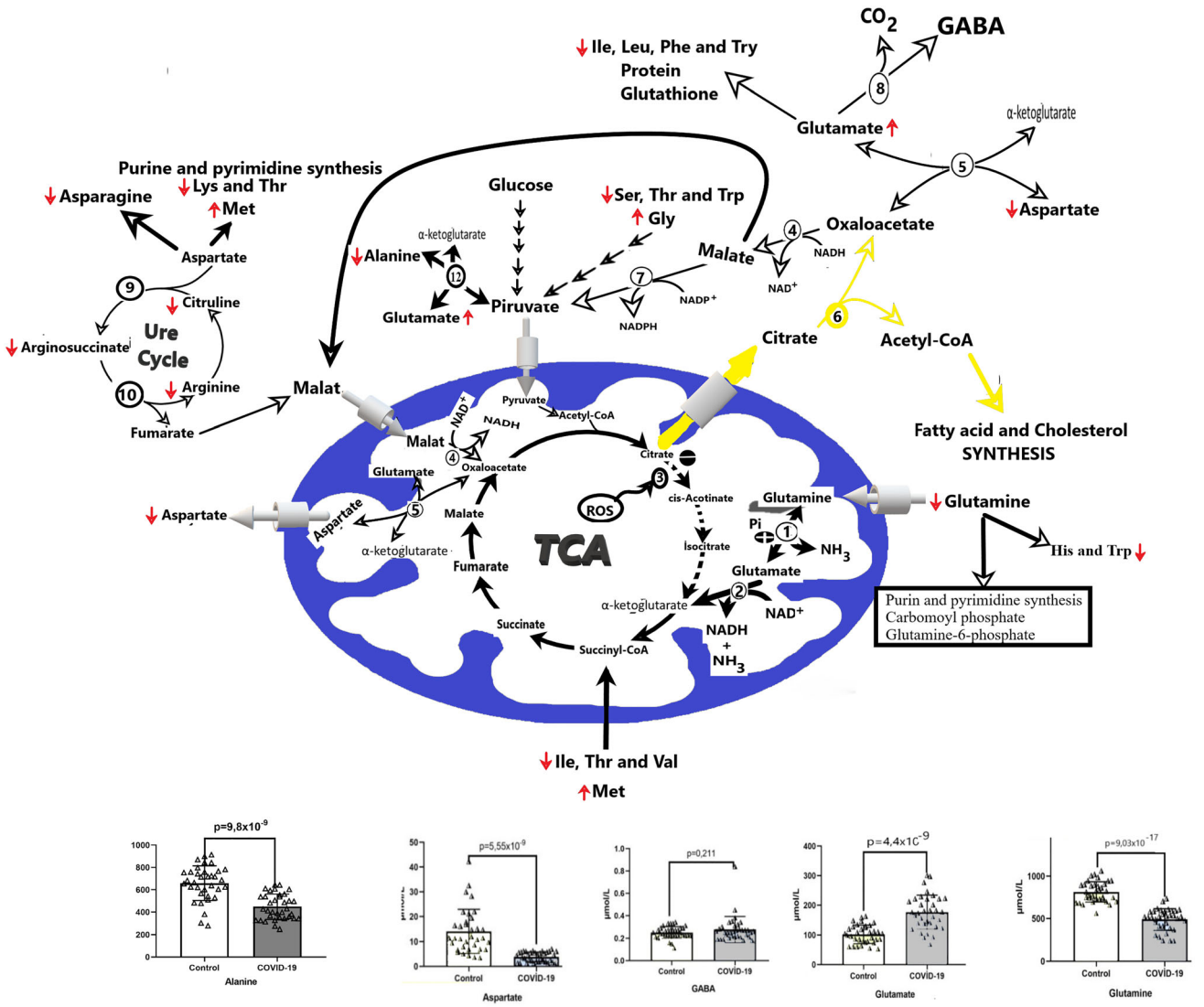

Fig. 2 Relationship of amino acid between glutaminolysis and malate-aspartate shuttle in COVID-19 patients. In viral infections, genome replication, the viral products accelerate glycolysis to support pathogen biosynthesis by providing glycolytic intermediates for the synthesis of nucleic acids, amino acids and lipids required for them, rather than energy. Mitochondrial carrier protein (MPCP). Notes: 1-Glutamine synthase, 2-Glutamate dehydrogenase (GDH), 3-Acotinase, 4-Malate dehydrogenase, 5-Aspartate transaminase (AST), 6-Malic enzyme, 8-Glutamate decarboxylase, 9-Arginosuccinate synthase (ASS), 10-Aginosuccinate lyase (ASL), 11-Alamine transaminase (ALT)

Glutamine also contributes to the synthesis of reduced glutathione (GSH) by being converted to glutamate by glutaminase [44]. The conversion of glutamine to glutamate and then to α-KG with the enzyme glutaminase (GLS), its use in the synthesis of energy and various biomolecules is called the glutaminolysis pathway (Fig. 2) [45]. Münger et al. found that ^{13}C-labeled glutamine was converted to α-ketoglutarate, citrate, and malate at a similar rate [34]. This supports the decrease in glutamine levels due to the activation of the glutaminolysis pathway in our patient group (Fig. 2).

The main function of glutaminolysis is to provide intermediate metabolites to the TCA cycle for cell growth [46]. It has been suggested that the glutamine derivative α-KG is an essential component for the survival of glutamine-dependent cells [44, 47, 48]. Glutamine, glutaminolysis, and glutathione are a few of the ways to deal with oxidative stress [49, 50]. Oxidative stress is triggered in infections caused by viruses such as HIV 1, viral hepatitis, herpes viruses, respiratory viruses, and Crimean-Congo hemorrhagic fever [51, 52]. Laforge et al. studies show that oxidative stress is also increased in COVID-19 patients [53].

Reduced glutamine levels in COVID 19 patients may have adverse consequences for the patients' prognosis. In COVID19 patients, depleted glutamine levels might be

trigger differentiation of myofibroblasts from fibroblasts and increase abnormal collagen synthesis and fibrosis in the lungs [54].

From the immunometabolic stand point, Sikalidis et.al. determined that glutamine metabolism plays an important role in immune systems [55]. As mentioned earlier, glutamine is required for immune cell proliferation and is involved in many metabolic pathways such as amino acid, nucleotide, ATP, NADPH synthesis. Macrophages are one of the immune cells that use extracellular glutamine [56]. Glutamine consumption during activation of macrophages is quite different in the pro-inflammatory (M1) and anti-inflammatory (M2) stages. Glutamine, which is involved in the TCA cycle in M1 macrophages, mainly promotes GABA shunt and succinate synthesis [57]. In viral infections, Ca^{2+} entry into the cell decreases with increased Cl output via the GABA receptor, where lung epithelial cells are activated by GABA. GABA can reduce inflammation and improve alveolar fluid clearance and lung functional recovery in rodent models of acute lung injury [58, 59]. A study of mice infected with SARS-CoV-2 showed that the course of the disease and the mortality were lower in those who started GABA therapy after inoculation of virus [60]. However, we could not find significant differences in GABA levels in the patient group we studied (Table 1).

We can assume that not only the warburg effect and glutaminolysis, but also in the patient group we examined, HBP pathways could also be active. Glutamine is needed for the biosynthesis of hexosamines from glucose. When glucose enters the cell, it first turns into fructose-6-phosphate, It then reacts with glutamine through the GFAT enzyme converted to glucosamine-6-phosphate [61].

In our study, in the COVID-19 PCR positive group, non-essential amino acids (Ala, His, Ile, Leu, Phe, Trp, Tyr) synthesized from glutamine, urea cycle intermediate metabolites (citrulline arginosuccinate, arginine) and glutamine itself were significantly had been decreased (Table 1). Laviada-Molina and colleagues suggested that the hexosamine biosynthesis pathway (HBP) is active in SARS-CoV-2 patients. As it is known, HBP can cause fatal results with its contribution to cell proliferation, hyperglycemia, increased virus replication and cytokine storm [62].

According to Zaho et al. suggested that increased HBP enzyme levels in a respiratory tract infection caused by human metapneumovirus (hMPV) and changes in the metabolic pathway during infection play an important role in the life cycle of the virus [63]. In our patient group, we speculate that decreased plasma glutamine levels ($p = 9.03 \times 10–17$) and increased glutamate levels ($4.4 \times 10–9$) in COVID-19 patients may be due to the active HBP pathway (Figs. 1, 2). We plan to study this issue in more detail in our future work.

If our hypothesis is correct, the decrease in plasma glutamine levels in COVID-19 patients may be due to both the GABA shunt and the activation of the glutaminolysis and hexosamine biosynthesis pathway. Although we did not perform these tests, when these three pathways were active in COVID-19 infection, purine, pyrimidine and fatty acid synthesis would increase and the replication of the virus would be supported. There is also a study supporting the fact of glutamine depletion in COVID-19 patients. On the other hand, Cengiz et al. showed that oral glutamine supplementation shortens the length of hospital stay and reduces the need for intensive care in patients with COVID-19 [64].

Of course, this study raises the question of whether glutamine depletion is a positive metabolic response to keep the host alive or a negative process that favors virus replication.

Conclusion

When the plasma amino acid levels of COVID-19 PCR positive individuals were examined, it was seen that Alanine, Arginine, Arginosuccinic acid, Aspartate, Glycine, Histidine, Lysine, Citrulline, Threonine and Glutamine levels were decreased compared to healthy individuals. It has been clearly shown that amino acids are consumed in large quantities in this disease. As a result of our study, we saw that the decrease in many amino acids together with the onset of this disease can lead to burnout. We made inferences that could explain this decrease in amino acid levels.

When the metabolic pathways of amino acids in this disease are followed and especially the excessive decrease in glutamine level is considered, we came to the conclusion that glutaminolysis, hexosamine and pentose phosphate pathways may be activated in this disease.

It is believed that determining the plasma amino acid levels of these patients and administering them enterally or parenterally will improve the length of stay and pathophysiology of the patients in the hospital and intensive care unit. Therapeutic administration of parenteral or enteral amino acids may have beneficial effects on recovery.

Limitations of the Study

In our study, we determined only plasma amino acid levels. We proceeded from the hypothesis that the plasma amino acids, which were found to be decreased, were overused. However, we do not know about the expression of enzymes that regulate this metabolism. We do not have a clear idea

of the nutritional content of the diet that COVID-19 PCR-positive vakas receive.

All of the patients were treated in clinics without mortality, and none of them were treated in intensive care units in a way that would make a difference in amino acid levels.

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Author contributions HA and SNH researched literature and conceived the study. YKT, GT, İK, SK and SY were involved in protocol development, gaining ethical approval, patient recruitment and data analysis. HA wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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Declarations

Conflict of interest The authors declare that there are no potential conflicts of interest regarding the research, authorship and publication of this article.

Ethical approval The ethics committee of Cumhuriyet University approved this study (REC number: 2020–04/02).

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